CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising an orthogonal glutamyl-tRNA (glutamyl O-tRNA), wherein the glutamyl O-tRNA comprises at least about a 50% suppression efficiency in the presence of a cognate synthetase in response to a selector codon as compared to the glutamyl O-tRNA corresponding to a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC) tRNA).

2. The composition of claim 1, wherein the glutamyl O-tRNA comprises a G:C base pair at position 10:28.

3. The composition of claim 1, wherein the selector codon is an amber codon.

4. The composition of claim 1, wherein the glutamyl O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC) tRNA), or a complementary polynucleotide sequence thereof.

5. The composition of claim 1, further comprising an orthogonal glutamyl aminoacyl-tRNA synthetase (glutamyl O-RS), wherein the glutamyl O-RS preferentially aminoacylates the glutamyl O-tRNA with a selected amino acid in response to the selector codon.

6. The composition of claim 5, wherein the suppression efficiency of the glutamyl O-RS and the glutamyl O-tRNA together is 10 fold greater than the suppression efficiency of the glutamyl O-tRNA in the absence of the glutamyl O-RS.

7. The composition of claim 5, wherein the glutamyl O-RS, or a portion thereof, is encoded by a polynucleotide sequence comprising in any one of SEQ ID NO.: 68 (*Af*), 72 (*Mm*), 74 (*Mt*) or 76 (*Ph*), or a complementary polynucleotide sequence thereof.

8. The composition of claim 5, wherein the glutamyl O-RS comprises an amino acid sequence comprising any one of SEQ ID NO.: 69 (*Af*), 73 (*Mm*), 75 (*Mt*), or 77 (*Ph*), or a conservative variation thereof.

9. The composition of claim 5, wherein the glutamyl O-RS is derived from an organism selected from the group consisting of: an *Archaeoglobus fulgidus (Af), a Methanosarcina mazei (Mm), a Methanobacterium thermoautotrophicum (Mt), and a Pyrococcus horikoshii (Ph).*

10. The composition of claim 1, wherein the glutamyl O-tRNA is derived from one or more archaeal tRNAs.

11. The composition of claim 10, wherein the one or more archaeal tRNAs comprise archaeal glutamyl-tRNA, or derivative thereof.

12. The composition of claim 1, comprising a cell.

13. The composition of claim 12, wherein the cell is an *E. coli* cell.

14. The composition of claim 1, comprising a translation system.

15. A cell comprising a translation system, wherein the translation system comprises:

an orthogonal glutamyl-tRNA (glutamyl O-tRNA), wherein the glutamyl O-tRNA comprises at least about a 50% suppression efficiency in the presence of a cognate synthetase in response to a first selector codon as compared to the glutamyl O-tRNA comprising or encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC) tRNA);

an orthogonal aminoacyl-glutamyl-tRNA synthetase (glutamyl O-RS); and,

a first selected amino acid;

wherein the glutamyl O-tRNA recognizes the first selector codon, and the glutamyl O-RS preferentially aminoacylates the glutamyl O-tRNA with the first selected amino acid.

16. The cell of claim 15, wherein the glutamyl O-tRNA comprises a G:C base pair at position 10:28.

17. The cell of claim 15, wherein the glutamyl O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC)), or a complementary polynucleotide sequence thereof, and wherein the glutamyl O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 69 (*Af*), 73 (*Mm*), 75 (*Mt*), or 77 (*Ph*), or a conservative variation thereof.

18. The cell of claim 15, wherein the cell further comprises an additional different O-tRNA/O-RS pair and a second selected amino acid, wherein the O-tRNA recognizes a second selector codon and the O-RS preferentially aminoacylates the O-tRNA with the second selected amino acid.

19. The cell of claim 15, wherein the glutamyl O-tRNA is derived from one or more archaeal tRNAs and the glutamyl O-RS is derived from an organism selected from the

group consisting of: an *Archaeoglobus fulgidus (Af), a Methanosarcina mazei (Mm), a Methanobacterium thermoautotrophicum (Mt), and a Pyrococcus horikoshii (Ph).*

20. The cell of claim 15, wherein the cell is a non-eukaryotic cell.

21. The cell of claim 20, wherein the non-eukaryotic cell is an *E. coli* cell.

22. The cell of claim 15, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises a selector codon that is recognized by the glutamyl O-tRNA.

23. An *E. coli* cell, comprising:

an orthogonal glutamyl tRNA (glutamyl O-tRNA), wherein the glutamyl O-tRNA comprises at least about a 50% suppression efficiency in the presence of a cognate synthetase in response to a selector codon as compared to the glutamyl O-tRNA comprising or encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC) tRNA);

an orthogonal glutamyl aminoacyl- tRNA synthetase (glutamyl O-RS), wherein the glutamyl O-RS preferentially aminoacylates the glutamyl O-tRNA with a selected amino acid;

the selected amino acid; and,

a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises the selector codon that is recognized by the glutamyl O-tRNA, and wherein the glutamyl O-tRNA is derived from one or more archaeal tRNAs and the glutamyl O-RS is derived from an organism selected from the group consisting of: an *Archaeoglobus fulgidus (Af), a Methanosarcina mazei (Mm), a Methanobacterium thermoautotrophicum (Mt), and a Pyrococcus horikoshii (Ph).*

24. An artificial polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising a nucleotide sequence as set forth in SEQ ID NO.: 67;

(b) a polynucleotide that is complementary to or that encodes a polynucleotide sequence of (a);

(c) a nucleic acid that hybridizes to a polynucleotide of (a), or (b), under highly stringent conditions over substantially the entire length of the nucleic acid;

(d), a polynucleotide that is at least 90% identical to that of a naturally occurring glutamyl tRNA and comprises a G:C base pair at position 10:28;

(e) a polynucleotide that is at least 98% identical to a polynucleotide of (a), (b), (c), or (d); and,

(f) a polynucleotide comprising a conservative variation of (a), (b), (c), (d), or (e).

25. A vector comprising or encoding a polynucleotide of claim 24.

26. The vector of claim 25, wherein the vector comprises a plasmid, a cosmid, a phage, or a virus.

27. The vector of claim 25, wherein the vector is an expression vector.

28. A cell comprising the vector of claim 25.

29. A method of producing a pool of tRNAs that are orthogonal to a selected cell (O-tRNAs), the method comprising:

aligning a plurality of glutamyl tRNA sequences;

determining a consensus sequence;

generating a library of mutant glutamyl tRNAs using the consensus sequence;

mutating an anticodon loop of members of the library of mutant glutamyl tRNAs to allow recognition of a selector codon, thereby providing a library of potential O-tRNAs; and,

subjecting to negative selection a first population of cells of a first species, wherein the cells comprise a member of the library of potential O-tRNAs, to eliminate cells that comprise a member of the library of potential O-tRNAs that is aminoacylated by an aminoacyl-tRNA synthetase (RS) that is endogenous to the cell, thereby providing a pool of tRNAs that are orthogonal to the cell of the first species.

30. The method of claim 29, wherein the plurality of glutamyl tRNA sequences are derived from an archaeal species.

31. The method of claim 29, wherein the plurality of glutamyl tRNA sequences are derived from a species other than the first species.

32. The method of claim 29, further comprising mutating a G10:U28 base pair to a G10:C28 base pair.

33. The method of claim 29, wherein the consensus sequences is determining by an alignment program.

34. The method of claim 29, further comprising subjecting to positive selection a second population of cells of the first species, wherein the cells comprise a member of the pool of tRNAs that are orthogonal to the cell of the first species, a cognate aminoacyl-tRNA synthetase, and a positive selection marker, to select or screen for cells that comprise a member of the pool of tRNAs that is aminoacylated by the cognate aminoacyl-tRNA synthetase and that shows a desired response in the presence of the positive selection marker, thereby providing an O-tRNA.

35. The method of claim 34, wherein the second population of cells comprise cells that were not eliminated by the negative selection.

36. An O-tRNA produced by the method of claim 29.

37. A method for identifying an orthogonal glutamyl-aminoacyl-tRNA synthetase for use with a glutamyl O-tRNA, the method comprising:

subjecting to selection a population of cells of a first species, wherein the cells each comprise:

1) a member of a plurality of glutamyl aminoacyl-tRNA synthetases (glutamyl-RSs);

2) the orthogonal glutamyl tRNA (glutamyl O-tRNA) derived from one or more species, wherein the glutamyl O-tRNA comprises at least about a 50% suppression efficiency in the presence of a cognate synthetase in response to a selector codon, as compared to the glutamyl O-tRNA comprising or encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC) tRNA); and,

3) a polynucleotide that encodes a selection marker and comprises at least one selector codon;

wherein cells that show enhanced suppression efficiency as compared to cells lacking or comprising a reduced amount of the member of the plurality of glutamyl-RSs that comprises an active glutamyl-RS that aminoacylates the O-tRNA;

comparing a level of aminoacylation by the active glutamyl-RS of a first set of tRNAs from the first species to the level of aminoacylation by the active glutamyl-RS of a second set of tRNAs from a species other than the first species; wherein the level of aminoacylation is determined by a detectable substance; and,

selecting the active glutamyl-RS that more efficiently aminoacylates the second set of tRNAs compared to the first set of tRNAs, thereby providing the orthogonal glutamyl-aminoacyl-tRNA synthetase for use with the glutamyl O-tRNA.

38. The method of claim 37, wherein the selection comprises a position selection and the selection marker comprises a positive selection marker.

39. The method of claim 37, wherein the plurality of glutamyl RSs comprise mutant glutamyl RSs, glutamyl RSs derived from one or more species other than the first species or both mutant glutamyl RSs and glutamyl RSs derived from a species other than the first species.

40. The method of claim 37, wherein the aminoacylation is in vitro.

41. The method of claim 37, wherein the aminoacylation is in vivo.

42. The method of claim 37, wherein the detectable substance is a labeled amino acid.

43. An orthogonal aminoacyl-tRNA synthetase identified by the method of claim 37.

44. A method of producing a protein in a cell with a selected amino acid at a specified position, the method comprising:

growing, in an appropriate medium, the cell, where the cell comprises a nucleic acid that comprises at least one selector codon and encodes a protein;

providing the selected amino acid;

wherein the cell further comprises:

an orthogonal glutamyl-tRNA (glutamyl O-tRNA) that functions in the cell and recognizes the selector codon; wherein the glutamyl O-tRNA comprises at least about a 50% suppression efficiency in the presence of a cognate synthetase in response to the selector codon as compared to the glutamyl O-tRNA comprising or encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC) tRNA); and,

an orthogonal glutamyl aminoacyl-tRNA synthetase (glutamyl O-RS) that preferentially aminoacylates the glutamyl-O-tRNA with the selected amino acid; and,

incorporating the selected amino acid into the specified position in the protein during translation of the nucleic acid with the at least one selector codon, thereby producing the protein.

45. The method of claim 44, wherein the glutamyl O-tRNA is derived from one or more archaeal tRNAs and the glutamyl O-RS is derived from an organism selected from the group consisting of: an *Archaeoglobus fulgidus (Af), a Methanosarcina mazei (Mm), a Methanobacterium thermoautotrophicum (Mt), and a Pyrococcus horikoshii (Ph).*

46. The method of claim 44, wherein the glutamyl O-tRNA comprises a G:C base pair at position 10:28.

47. The method of claim 44, wherein the glutamyl O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in SEQ ID NO.: 67 (AE(GC)).

48. The method of claim 44, wherein the cell is a non-eukaryotic cell.

49. The method of claim 48, wherein the non-eukaryotic cell is an *E. coli* cell.